



**Naval Medical Research Center  
Biological Defense Research  
Directorate**

**Biological Warfare Agent Detection  
at BDRD: Past and Future**

**Robert Bull Ph.D.**



# Biological Defense Research Directorate (BDRD)

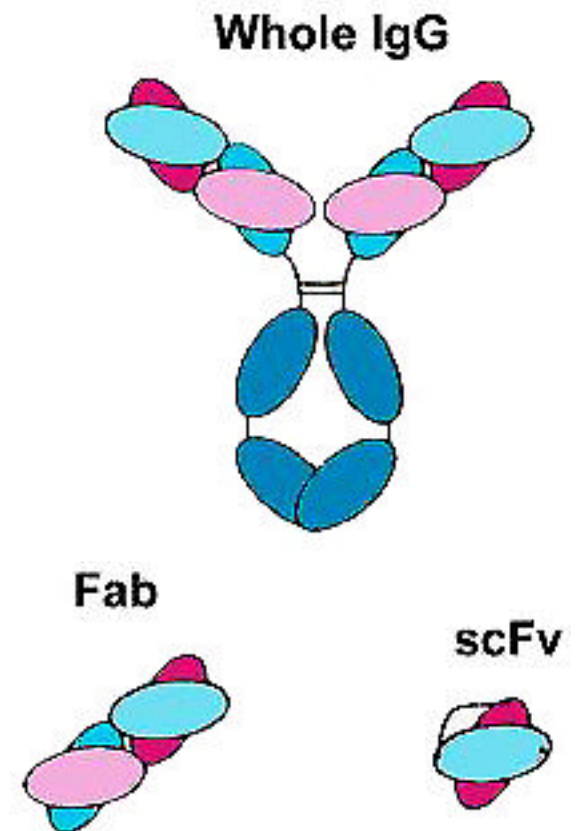
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- Vaccine
- Molecular Diagnostics
- Microbiology
- Sample Management Facility
- Deployable Laboratory
- Immunodiagnostics



# Molecular Diagnostics

- Recombinant antibodies
- PCR protocol development
- Recombinant antigens
- Vaccine and antibody development





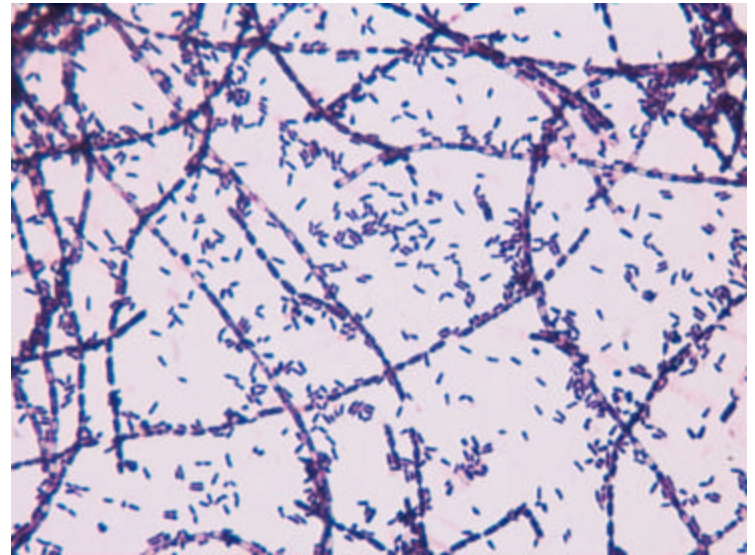
# Molecular Diagnostics

- Initial PCR protocol development with Robocyclers, PE480 and IGEN
- First deployed military PCR capability in Desert Storm/Desert Shield
- Transitioned to Taqman and deployable platforms as available
- Current suite of 45+ Taqman assays for 20+ BW agents



# Microbiology

- BSL-3 Facility
  - Antigen production
  - Nucleic acid purification
  - Specialty analysis
  - Genomics

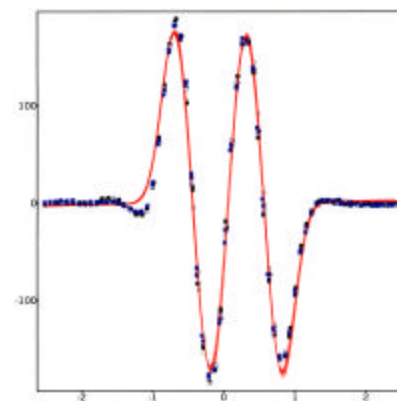
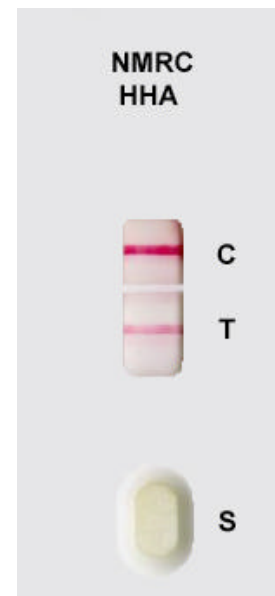


- Sample Management Facility



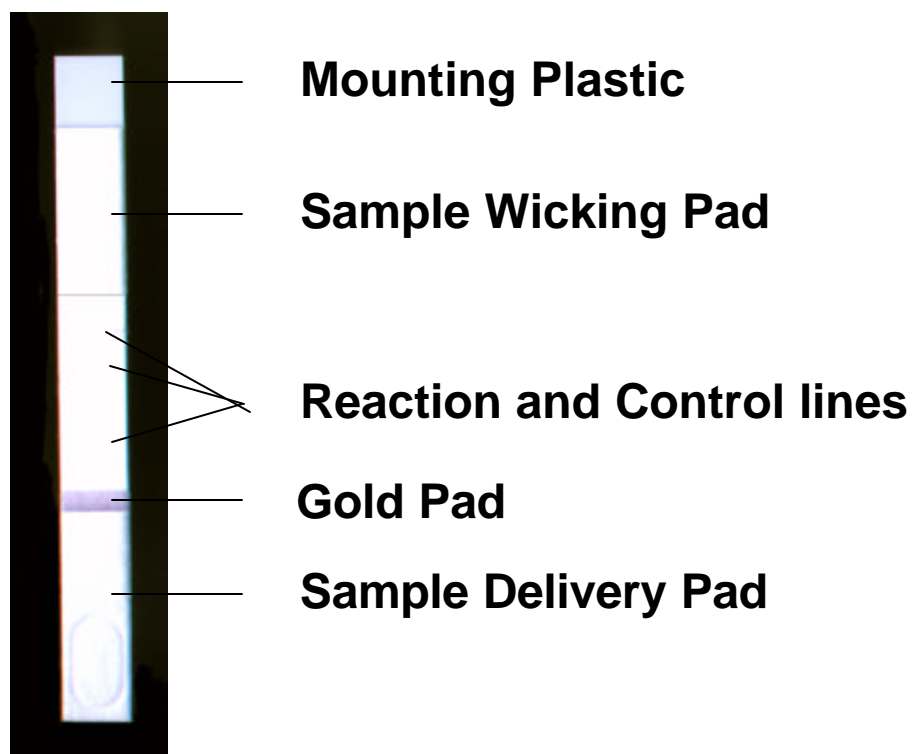
# Immunodiagnosics

- Reagent Development
- Assay Development  
ELISA, ORIGIN, TRF
- Platform Development  
CANARY B CELL, FC
- Rapid Hand-Held Assays  
Colloidal Gold  
Paramagnetic Beads





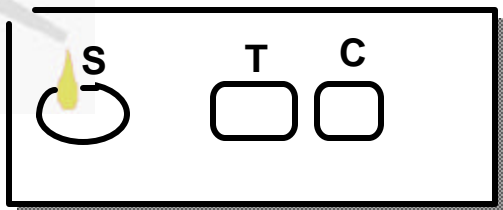
# Immunochromatographic Assay Strip



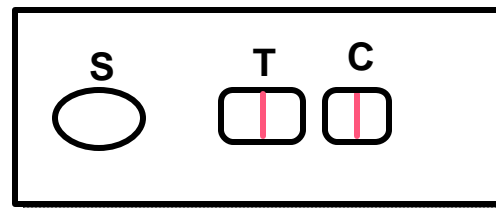
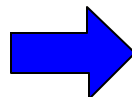


# One Step Hand-Held Assay (BW Agent Detection)

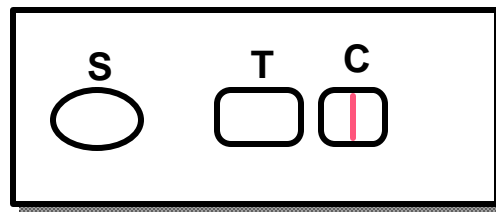
**Add Sample to Device**



**Serum, Urine, Saliva, Collected Air or  
Other Environmental Samples**



***Positive***



***Negative***

**Read after Fifteen Minutes**





# Background on NMRC Hand-Held Assays

- Currently there are 28 different assays against many different agents (Bacterial: Vegetative and Spore, Viral agent, and several Toxins) that have been developed at BDRD.
- The mass production is now done by JPO Bio however most reagents and assays were originally developed at Naval Medical Research Center in the Biological Defense Research Directorate.
- One current goal is to develop assays with a dramatic (at least 1 log) increase in sensitivity without any loss in the convenience of the current Hand-Held assay format.



# Why is the Increased Sensitivity Needed

- Toxins are highly lethal molecules; Botulinum toxin type A is 100,000 times more lethal than Sarin (organophosphate nerve agent used by Alum Shinrikyo cult in Japanese subway attack)
- Current methods are effective at detecting in the ng/ml levels. **Problem:** low level contamination that is potentially lethal could be missed.
- PCR while very effective and sensitive, is not very helpful at detecting toxins.



# How to Increase Sensitivity

- New better reagents
  - Time consuming and potentially costly(work is underway)
- Use available technologies
  - Consider technologies under development (tech-watch)
  - Image analysis (the human eye is pretty good).
  - Up converting phosphors (if the reader fails the strip give no signal).



# How to Increase Sensitivity cont.

- Magnetic particle attached to antibodies
  - No loss in sensitivity when compared to current Hand-Held assays with out using a reader. The line will appear brown (paramagnetic particle attached to antibody) instead of red (80nM gold particle attached to antibody)
  - Problem that needs to be overcome
    - Source and physical characteristics of particles.
    - Conjugation chemistry optimization
    - Laboratory vs Field readers (Quantum Design?)

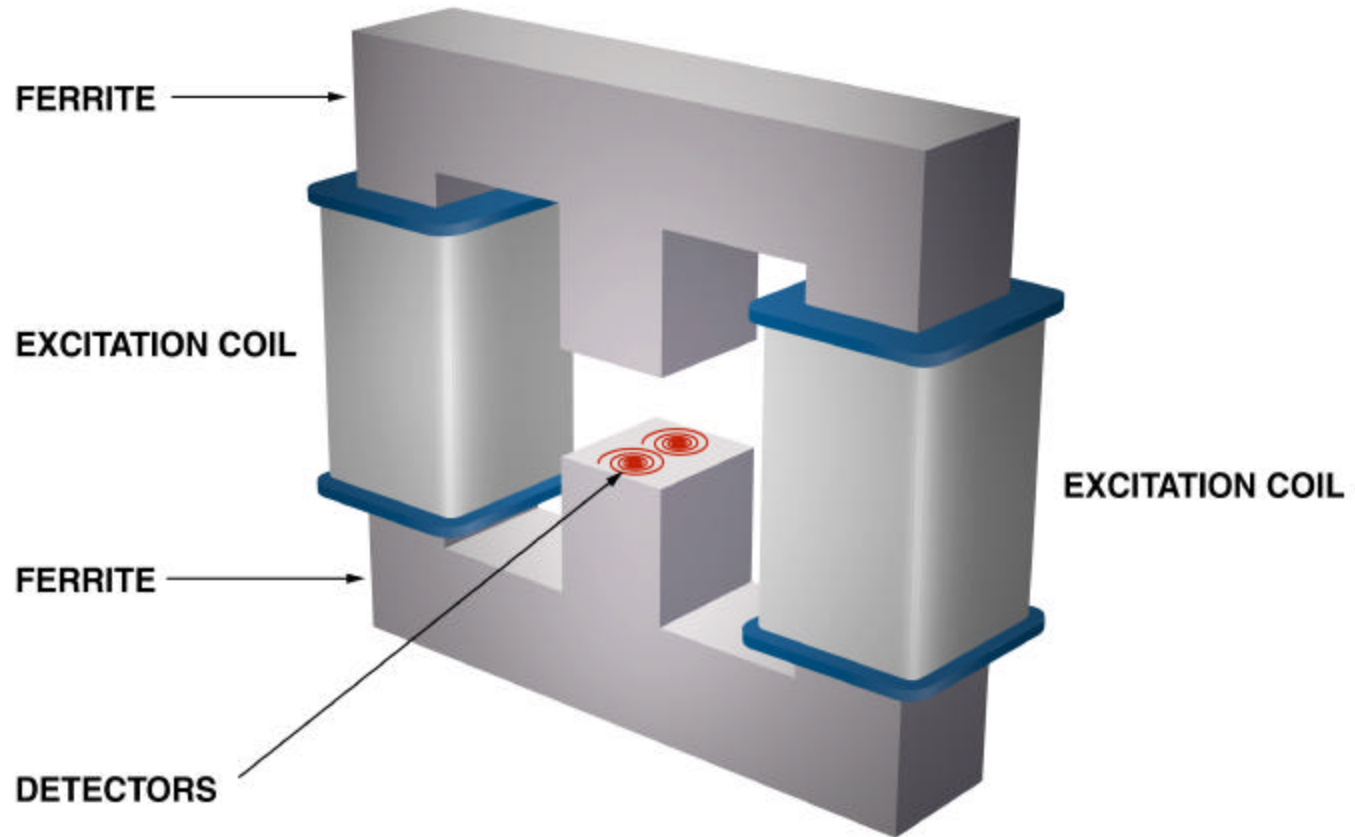


# Current Development work at BDRD

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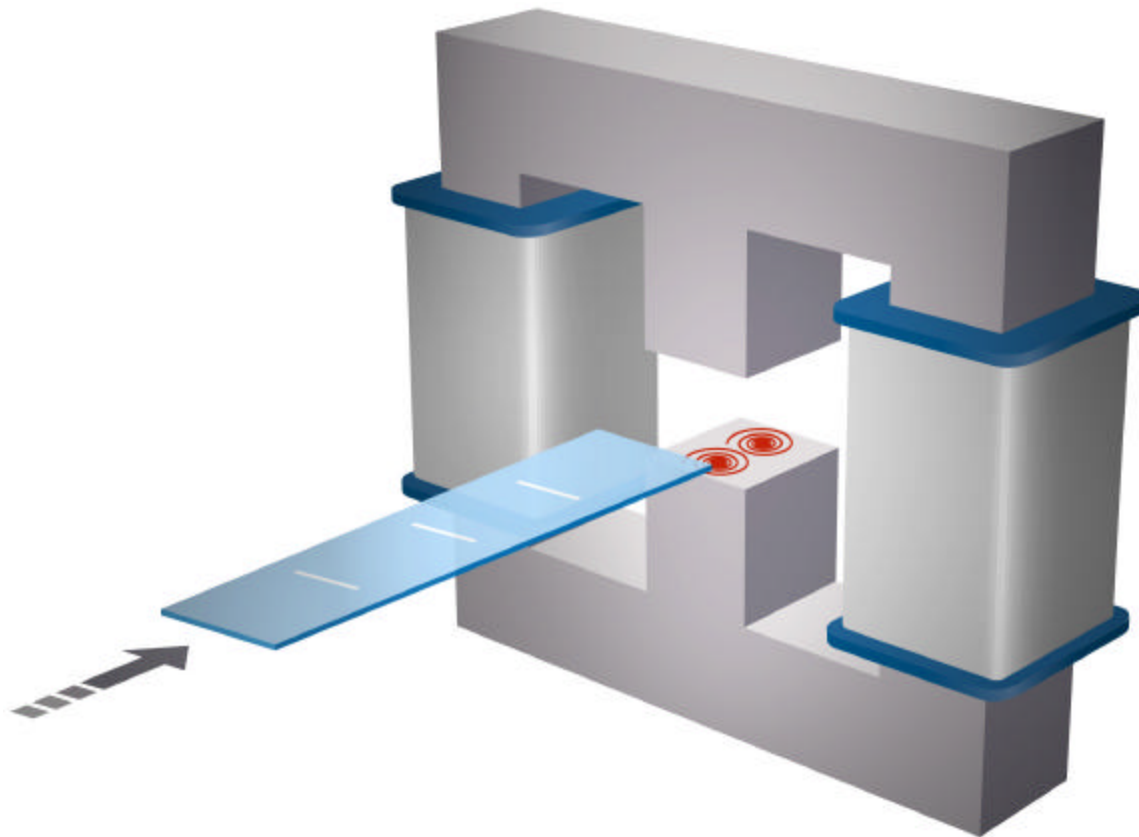
- Using the (Magnetics Assay Reader)MAR 3 developed by Quantum Design prototype assays have been developed.
- Conjugation protocol are being developed at BDRD as well as evaluations of custom conjugations for various companies

# The Heart of the Detection System



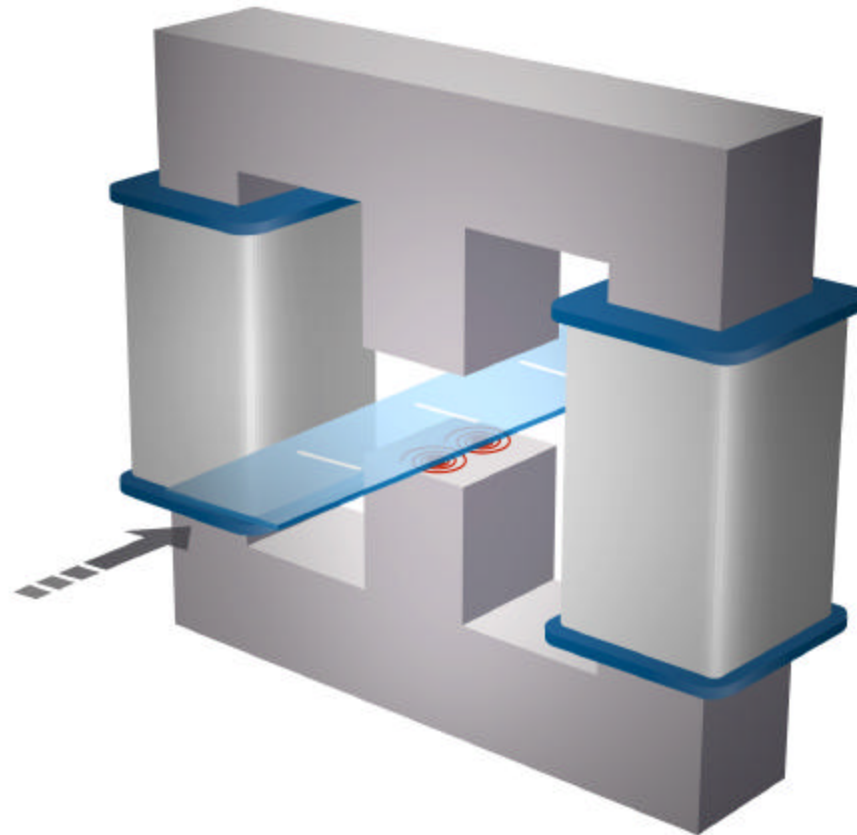
# The Gap and Lateral Flow Assay Design

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# The Gap and Lateral Flow Assay Design

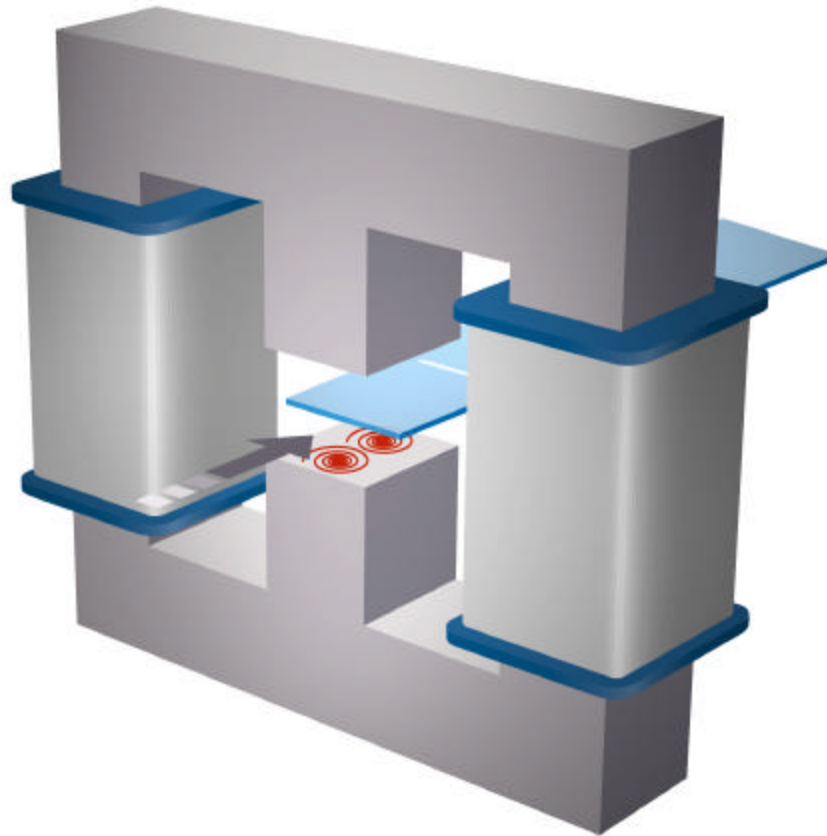
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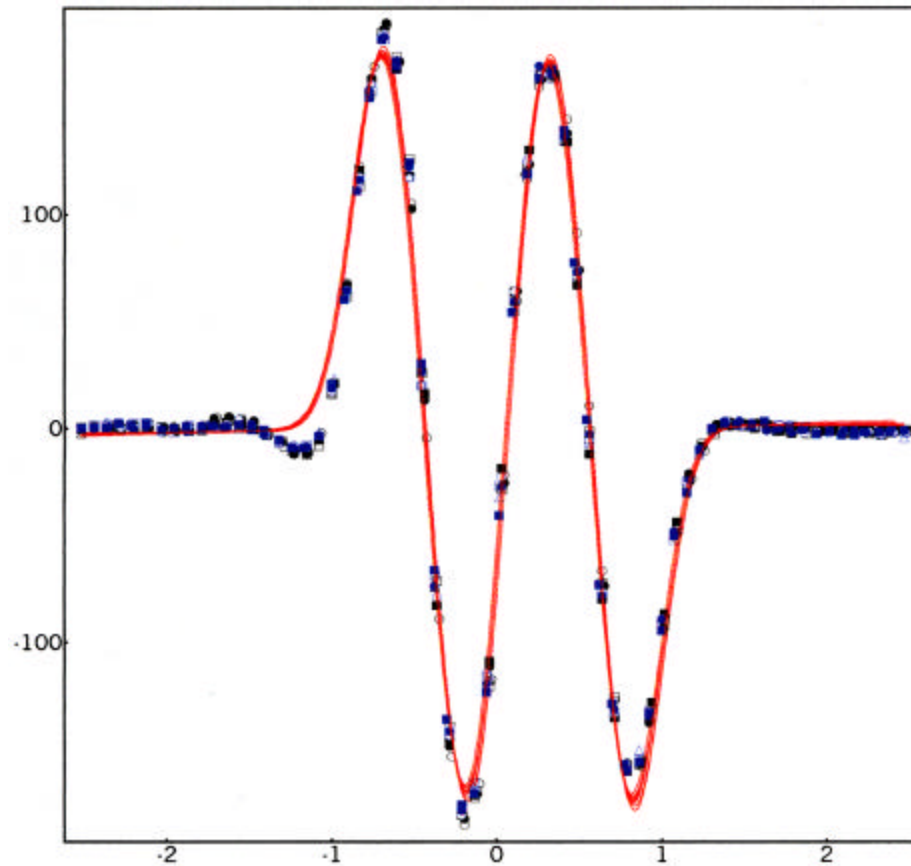


# The Gap and Lateral Flow Assay Design

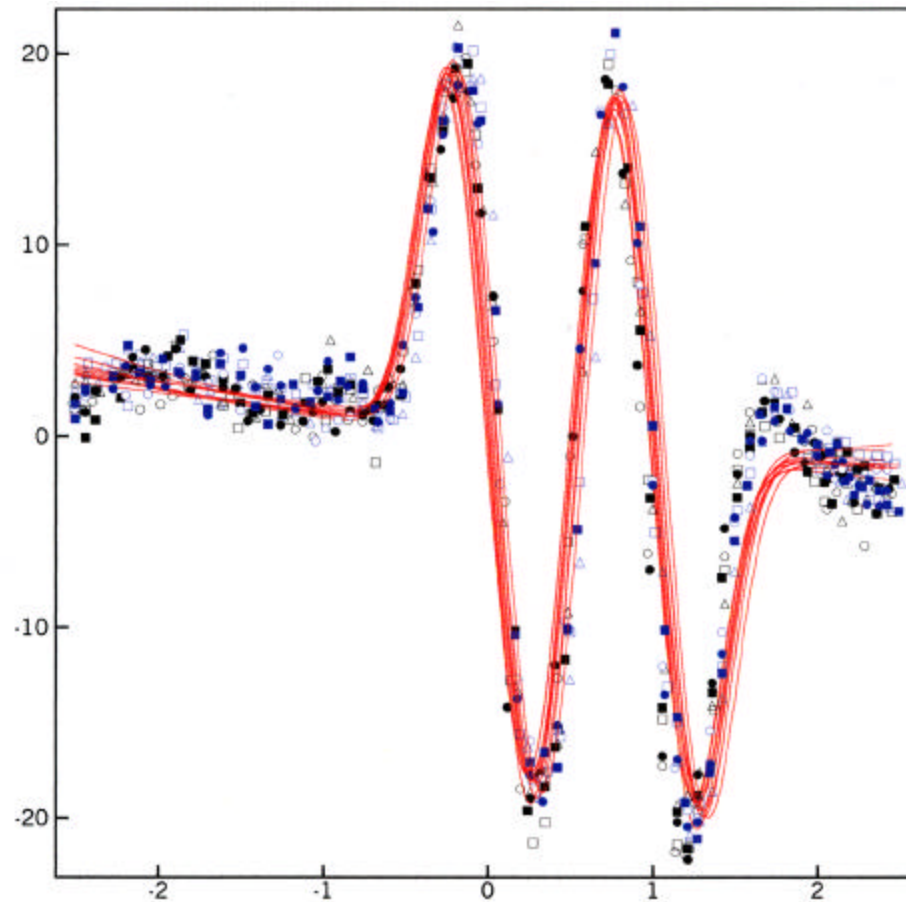
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# The Signal

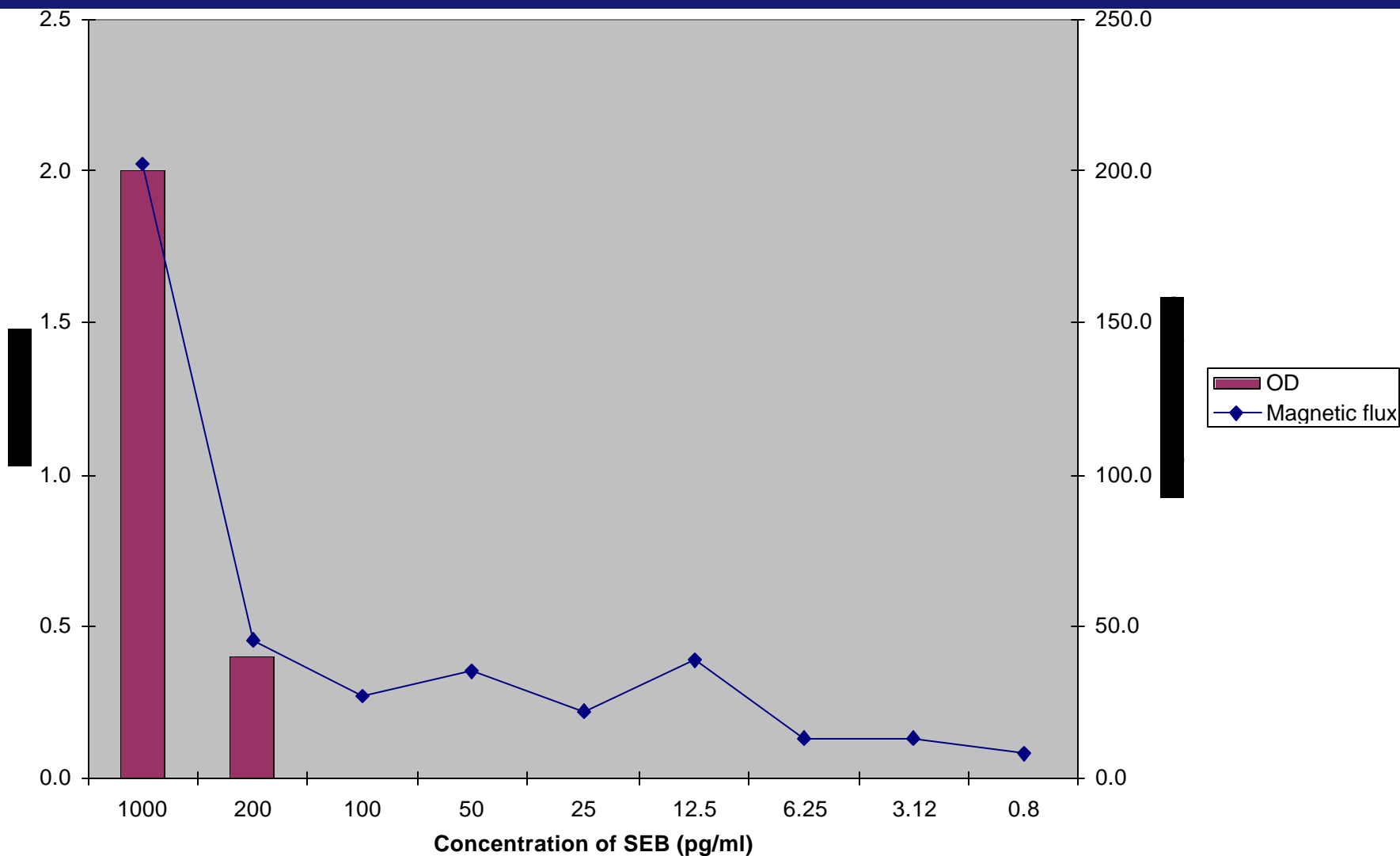


# 10 femtograms/ml





## Comparison of Magnetic and Gold Labeled SEB Immunochromatographic Assays





# Conclusions

- Magnetic reader technology can give a 1 to 3 log increase in sensitivity using currently available reagents and platform.
- This sensitivity is critical for the detection of toxins.
- Stable particles and conjugation protocols are needed for assay development.
- Mass production will be needed to get this technology to the end users.